CHAPTER IV
EXPERIMENT 2
INDUCTION OF MULTIPLE FOLLICULAR DEVELOPMENT AND OVULATION IN THAI-NATIVE GOAT USING FSH AND hCG

1. Introduction

The demand for goat meat in Thailand has been increasing especially in the southern area with ethnic populations. Goat play an important role as an integral part of farming and social and religious especially for Thai-Muslim people. Although the goat potentially is a highly prolific animal which has a relatively short gestation period compared to other livestock species, little producer is known about the reproductive management due to a lack of information on the reproductive management i.e., method of mating, time of first mating, pre- and postpartum management (Kochapakdee et al., 1995). In addition, a lack of knowledge in the management application and assortments of reproductive technology for Thai-native goat production is another major concern. These limitations therefore will mostly affect the efficiency and capacity of goat production.

Recently, tremendous developments have been achieved in the area of assisted reproductive technology (ART) both in humans and animals in the last two decades. In livestock including goats, the modern ART are being used for the improvement and preservation of livestock genetics and the enhancement of reproductive efficiency. The ART includes artificial insemination, embryo transfer, estrus synchronization and superovulation, multiple ovulation embryo transfer, laparoscopic ovum pick-up, *in vitro* production of embryos, intracytoplasmic sperm injection, cryopreservation of sperm, cryopreservation of oocytes and embryos, sexing of sperm and embryos, embryo splitting, cloning and gene transfer and marker-assisted selection. Goat is an excellent model for all these ARTs and has been used extensively in both basic and applied research. The applications of these ARTs in goats enable to increase the rate of genetic progress, reduce generation interval, enhance production, help utilize

genetically important but biologically inferior individuals, improve the management of infertile/sub-fertile buck/doe and eliminate reproductive diseases.

Protocols for multiple ovulation are widely used to improve the number of offspring from selected female goats (Baril and Saumande, 2000), as in other ruminant species. However, similar to cows or sheep, the high variability in the number of corpora lutea and embryos obtained in response to superovulation, between treatments and between individual animals in the same treatment group, is a major limiting factor in goat embryo transfer programs. This variability, due to both extrinsic factors-source, purity of gonadotropins and protocol of administration and intrinsic factors-breed, age and reproductive status (Holtz, 2005), still remains despite refinements in the use of new gonadotropin preparations and animal management systems (Tibary et al., 2005).

In goats, superovulatory treatment typically consists of a combination of estrous cycle control (usually involving application of progestagen implants) with an elevated dose of a gonadotropin, to induce the ovary to release more than the typical number of oocytes. The use of eCG with or without a follow-up with eCG antibodies (Pintado et al., 1998), in many cases did not deliver the anticipated response. This might be associated with the rapid degradation of eCG in goats; its half-life being only 10–15 h, which is several times shorter than in cows. Follicle stimulating hormone, usually of porcine origin (pFSH), proved to be more efficacious than eCG (Nowshari et al., 1992), provided it contains an appropriate admixture of luteinizing hormone (LH). Nowshari et al. (1995) reported that LH content in the range of 40% does not only provide the best superovulatory response but also superior embryo viability. Since the half-life of pFSH in goats is only 5 h (Demoustier et al., 1988), FSH is administered twice daily for 3–4 days, usually in a decreasing dosage, beginning between 1 and 3 days before the end of the progestagen treatment. On average 8–16 ovulations are generated, although individual variability is immense. Several attempts have been made to devise less labour-intensive treatment regimes without compromising embryo yield. One such attempt was to inject FSH at 24 instead of 12 h intervals while doubling the dose. This resulted in an average ovulation rate of 8.9 as compared to 10.8 in a control group submitted to conventional

treatment (Holtz, 2005). This treatment saves on labour and expense and has been adopted by a number of commercial embryo transfer operations.

The objective of the present study was to evaluate induction of multiple follicular growths and ovulation in Thai-native goats treated with FSH and hCG (FSH decreasing dose, 2 or 3 days protocols).

2. Materials and Methods

2.1 Animal Ethics

Experiment protocols were approved by the animal ethics committee of Khon Kaen University (No. AEKKU 13/2551; March 25th, 2008).

2.2 Animals and design

The study was conducted during the rainy season at the experimental farm of the University. The University is located at 102 degrees east longitude and 16 degrees north latitude with a tropical climate. The experiment was carried out at the experimental farm, the small ruminant unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University during June to October 2009. Sixteen nulliparous Thai-native does with an average age and body weight of 14 months and 21±0.6 kg, respectively. Animals were routinely assessed for estrous activity by exposing all does to a vasectomized buck. The estrus was detected and designed as day 0. Animals were then randomly assigned to one of two treatments goats: FSH treatment for two days (2D-FSH) (FSH-P; Folltropin-v®, Bioniche Animal Health, Canada), or FSH treatment for three days (3D-FSH). Group 2D-FSH was intramuscularly injected twice daily FSH for 2 days (5, 4 units per injections; 18 mg), starting on Days 18, 19 and with 300 IU hCG (Chorulon®, Intervet, UK) on the morning Day 20 of estrous cycle. Group of 3D-FSH was injected twice daily with FSH for 3 days (5, 4, 3 units per injections; 24 mg), starting on day 17, 18, 19 and with hCG on day 20 of the estrous cycle. Animals were fed with roughage, clean water whereas mineral block was provided for ad libitum comsumption. The concentrate (16% CP) was fed at 1% of body weight.

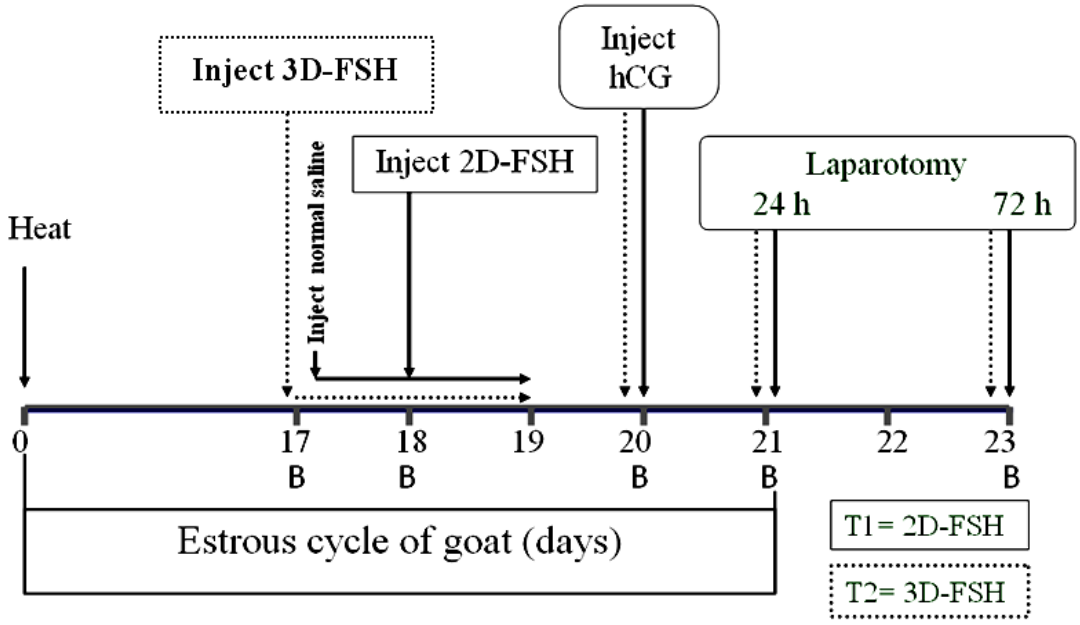

Figure 4.1 Description of timing of injections for the two treatments FSH protocols, laparotomy for detect to corpora hemorrhagica (CH) at 24 h and at 72 h to count corpora lutea (CL)

2.3 Data collection

Data for this study were collected from June to October 2009. Goats laparotomy was performed according to the standard procedure described by Jarell and Dzik (1991) after hCG injection to evaluate number of corpora hemorrhagica (CH) and /or corpus luteum (CL). Ovulation rates calculated by numbers of CH expressed as a percentage of the total number of CL (Stenbak et al., 2003). Follicular growths determined by number and size of follicle also evaluated according to Gonzalez-Bulnes et al. (2003). Blood samples were taken to determine plasma progesterone (P4) concentration on day 17 (group of 3D-FSH) or day 18 (group of 2D-FSH), day 20 (before hCG injection) day 21 (laparotomy at 24 h) and day 23 (laparotomy at 72 h). Immediately after collection, blood samples were centrifuged at 1500×g for 15 min. The plasma was aspirated into vials and then stored at -20^{o}C until assayed for P4. In addition, other characteristics such as BW and BCS. Body condition scores were assessed by the herd manner at laparotomy to determine the

effect of BCS on response variables. Score was assigned to each goat using a quarter-point scale from 1 to 5, where 1 = emaciated and 5 = obese (Ferguson et al., 1994).

2.4 Hormone assays

Plasma progesterone concentrations were determined by competitive ELISA (Crane et al., 2006). Goat anti-mouse IgG (H+L) was made in mouse by using a P4-horse radish peroxidase conjugate. Intraassay coefficient of variation was 2.65%, and assay sensitivity was 0.025 ng/ml.

2.5 Statistical analyses

The statistical model included treatment, the ovulation rate at 24 h (OR) and BCS at the beginning of trial as regression variables. The effect of OR and BCS were converted to categorical variables by grouping goats by ovulation rate at 24 h and BCS at initial trial (SAS, 2000). Treatment interactions the OR, and BCS were then reanalyzed using Chi-square analysis (Cochran-Mantel-Haenszel) of SAS. Continuous data (CH, CL, Follicle and P4) were analyzed using procedure GLM of SAS. Plasma P4 concentrations were analyzed with a nested analysis of variance with treatment, animal (treatment), and day included in the model, and differences between specific means were evaluated by using the student *t*-tests (SAS, 2000).

3. Results and Discussion

3.1 Superovulatory response

Characteristics of body condition score (BCS) in goats were not different between 2D-FSH and 3D-FSH groups in this study (2.87±0.07 vs 2.93±0.06 respectively, $P>0.05$). Ovarian follicular development of 2D-FSH was evaluated from the numbers of CH by laparotomy at 24 h as 2.0±0.40 and number of CL at 72 h as 5.0±0.70 ($P<0.05$), ovulation rate by laparotomy at 24 h was 40% (Table 5.1). In 3D-FSH group, the numbers of CH, CL at 24 and 72 h were 4.75±1.03 vs 8.5±1.04 respectively ($P<0.05$) same as 2D-FSH group. Moreover, ovulation rate was 56% during at 24 h (Table 4.1)

Table 4.1 Number of CH, CL and percentage of ovulation at different times responding FSH and hCG treatments.

Items	2D-FSH	3D-FSH	P-value
Number of CH at 24 h	2.00±0.40[a]	4.75±1.03 [b]	0.04
Number of CL at 72 h	5.00±0.70 [a]	8.50±1.04 [b]	0.03
Ovulation rate at 24 h (%)	40[c]	56 [d]	0.01

[a,b] Means±SEM differ within row, P<0.05

[c,d] Percentages of ovulation differ within row, P<0.01 (Chi-square test)

Number of CH of goat receiving 2D-FSH was less than goat receiving 3D-FSH (2.0±0.40 vs 4.75±1.03 respectively; P<0.05). However, the number of CL in 2D-FSH was less than 3D-FSH group at 72 h (5.0±0.70 vs 8.5±1.04; P<0.05) and ovulation rate of 2D-FSH group were same too. In addition, number of CH, CL and percentage of ovulation at 24 and 72 h after induced by FSH show that in Table 4.1. Ovulation rate of all groups during at 72 h were as 100% because of found only CL on ovaries, but there were not expressed of CH. Moreover, in this study using hCG to manipulated of limit timing in ovulation within 72 h.

In this study, the goats of 2D-FSH group was distinctly response on FSH less than 3D-FSH group, such as number of CH at 24 h, number of CL at 72 h and ovulation rate in 2D-FSH group. Body condition scores were assessed by the herd manner (BCS = 2.5-3.0) to determine the effect of BCS on response ovulation rate, no effect on present study.

3.2 Effect of FSH and hCG on follicular growth

Numbers of follicles in class 1-3, 4-6 and ≥7 mm at 24 h were not sigificantly different between 2D-FSH and 3D-FSH group. However, the numbers of follicles in class 4-6 mm of 2D-FSH were greater than that the 3D-FSH group during at 72 h (1.75±0.47 vs 0.25±0.25; P<0.01) but in the class ≥7 mm of 3D-FSH were greater than the 2D-FSH group (P<0.01); presented in Table 4.2.

Table 4.2 Number of follicular size 1-3, 4-6, and ≥7 mm in FSH treated goats.

Size of follicle	2D-FSH (n=8)	3D-FSH (n=8)	P-value
1-3 mm	0.50±0.28	1.00±1.00	0.08
4-6 mm	1.75±0.47 [a]	0.25±0.25 [b]	0.01
≥7 mm	2.75±1.03 [a]	6.00±1.22 [b]	0.01
Mean ± SEM	5.00±0.70	7.25±0.83	0.06

[a,b] Means±SEM differ within row, P<0.01

Protocols for induction of multiple follicular growth and superovulation currently used in the animal industry are not fully optimized (Gordon, 1997). The goat semi-industry has been reluctant to commercialize embryo transfer and other reproductive technologies because of the inconsistency of ovarian response to the superovulatory treatments (Cognie, 1999). It is known that ovarian stimulation with gonadotropins has an effect on oocyte maturation and competence. The FSH is the gonadotropins most frequently used, providing the best results in small ruminants. Both in sheep and in goat, FSH treatment results in high ovulation rate and better quality embryos per donor than treatment with PMSG. Currently, FSH is administered at 12 hours intervals in decreased or constant doses for 3-4 days starting two days before sponge withdrawal or implant removal, and around the time at which prostaglandin F2α is administered. The dose of total FSH administered varies from 16 to 20 mg depending on type of FSH preparation and genetic features of the sheep and goats donors (Baril et al., 2000). However, the objectives of this study were to optimize the FSH decreasing dose, 2 or 3 days can be an effective protocols for goats expecting that these appropriate technologies and could be used enhance the efficiency of goat production.

Follicle stimulating hormone (FSH) was shown to induce development of multiple follicles on each ovary when injected into goats or sheep for two or more days at regular intervals during the normal breeding season and non-breeding season (Gordon, 1997; Stenbak et al., 2001). Previous studies have shown that exposure of oocytes to various hormones in vivo cause maturational changes that are necessary for

proper development to occur (Armstrong et al., 1994; Stenbak et al., 2001). Optimal levels of exogenous gonadotropins should be used to promote proper oocyte development and depending on the regime of gonadotropin treatment, positive or negative effects on oocyte maturation and fertilization have been observed (Stenbak et al., 2001).

The results from this study demonstrate that is ovulation rate of all groups during at 72 h were as 100% because of found only CL on ovaries, but there were not present of CH. Moreover, in this study using hCG was similar as LH to manipulated of limit timing in ovulation within 72 h. Similarly with Walker et al. (1986) using FSH or PMSG with GnRH induced of multiple follicular development and ovulation in Marino ewes, found that ovulation rates were as 79% within during at 54-66 h in ewes.

In this study, the goat of 3D-FSH group was distinctly response on FSH greater than 2D-FSH group, such as number of CH at 24 h, number of CL at 72 h and ovulation rate in 3D-FSH group. Body condition scores were assessed by the herd manner (BCS = 2.5-3.0) to determine the effect of BCS on response ovulation rate, no effect on present study. Moreover, the Thai-native goats adequate responded to FSH treatments by exhibiting greater than or equal to four CL (2D-FSH = 5.0±0.70, 3D-FSH=8.50±1.04 CL) but less than to four CL were not responding on FSH (Stenbak et al., 2003). However, Cognie (1999) reported that about 20% of ewes did not respond to superovulatory procedures.

The ovulation rate was positively affected by a high number of follicles with a diameter of 1–6 mm at the onset of the treatment and negatively affected by a high number of follicles with a diameter ≥7 mm. Ovulatory follicles in superovulated does are derived from smaller antral follicles present at the onset of the FSH treatment which are able to respond of exogenous FSH. On the other hand, the initiation of the FSH treatment after the selection of the large dominant follicle could result in a lower ovulatory response, as in cows (Guilbault et al., 1991) or ewes (Gonzalez-Bulnes et al., 2003).

The response of goats, like other mammals, to treatments for the induction of superovulation and embryo production is strongly influenced by the structures (follicles and corpora lutea) present in the ovaries at the beginning of the exogenous

gonadotropin regimen. Thus, the ovulation rate at the end of the superovulatory treatment is related positively to the number of small gonadotropin-responsive follicles (2–3 mm in size) at first FSH dose (Gonzalez-Bulnes et al., 2003). On the other hand, the ovulation rate is affected negatively by the presence of a large dominant follicle (7 mm in size) in superovulatory protocols with a single dose of eCG or FSH (Lopez-Sebastian et al., 1999).

3.4 Progesterone concentration

In the present study, the plasma P4 concentrations in all groups were high on day 17, 18 (Mean±SE, 3.24±0.30 and 3.49±0.12 ng/mL respectively) then sharply decreased on day 20, after that to lowest on day 21, there were not different between groups (P>0.05). However, there were different in the plasma P4 concentrations within groups before being injected with FSH and after on day 20, 21 and 23 (P<0.05; Figure 4.2).

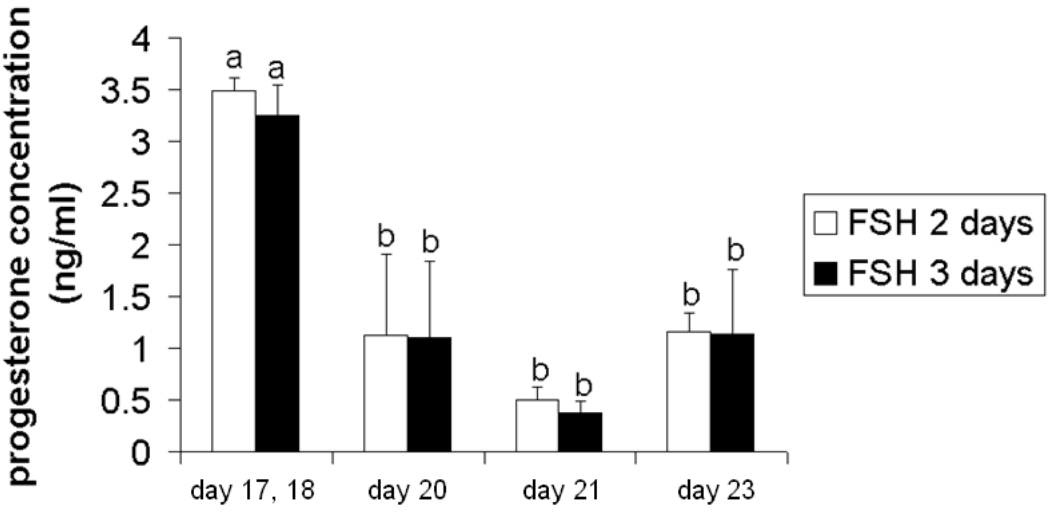

Figure 4.2 Mean (±SE) serum P4 concentrations (ng/ml) of goats on days prior injected 2D-FSH (day 18) 3D-FSH (day 17), hCG (day 20), laparotomy at 24 h (day 21) and 72 h (day 23).

a, b Proportion was differ (P<0.05)

Early findings in goats showed that follicular waves developing under continuous and high progesterone concentrations have smaller follicles than those developing when progesterone concentration are low (Rubianes and Menchaca, 2003). In the present study, the plasma P4 concentrations in all groups were high on days 17 and 18 then decreased on day 20, after that to lowest on day 21 confirmed this relationship between progesterone concentrations and the number of follicles development. Moreover, previous studies show FSH surges necessarily precede the emergence of a follicular wave in heifers (Adams et al., 2008), and a similar association of both events has been reported in ewes (Souza et al., 1997), but we did not measure FSH levels in the present study. According to Baird et al. (1992), FSH secretion was not affected directly by progesterone but was regulated by estradiol and inhibin, which was produced mainly by the largest follicles that developed during the cycle.

4. Conclusions

In summary, the majority of ovulation in Thai-native goat using FSH and hCG occurred between 24 and 72 h. These results indicate that superovulation with a decreasing dose of FSH (3D-FSH) and 300 IU hCG can be an effective protocol for Thai-native goat superovulation.